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Karyotypic studies in *Arachis hypogaea* L. varieties

LAVIA GRACIELA INÉS [1,2,*] and AVELIANO FERNÁNDEZ [1,3]

[1] Instituto de Botánica del Nordeste, C.C. 209, (3400) Corrientes, Argentina.
[2] Facultad de Ciencias Agrarias (UNNE).
[3] Facultad de Ciencias Exactas y Naturales y Agrimensura (UNNE).

Abstract — The karyotypes of nine *Arachis hypogaea* accessions, including two botanical varieties of subspecies *hypogaea* and four of subspecies *fastigiata*, were analyzed. Varieties *aequatoriana* and *peruviana* were studied for the first time. The mean chromosome length showed that variety *hirsuta* is distinctive when compared with the others because it is the shortest; besides, significant intravarietal differences exist within variety *hypogaea*, but not between subspecies. The karyotypes are highly symmetric since metacentric chromosomes are the most frequent. A pair of satellites was observed in all the accessions in mitotic metaphases, this feature was useful to differentiate groups. These data suggest that all *Arachis* accessions present a very similar chromosomal complement and support the hypothesis of a monophyletic origin.

Key words: *Arachis hypogaea*, chromosomes, karyotypes, peanut, varieties.

INTRODUCTION

The cultivated peanut, *A. hypogaea* (2n=40), one of the most widely cultivated grain legumes in the world, is a segmental allotetraploid (STEBBINS 1957) grown in tropical and temperate regions. Peanuts are rich in oil and protein content making a major contribution to human nutrition. According to the presence of flowers in the central axis, the species has been subdivided into two subspecies: subsp. *hypogaea* and subsp. *fastigiata* Waldron. Within the subspecies there are varieties as follows, subsp. *hypogaea*: var. *hypogaea* and var. *hirsuta* Köhler, subsp. *fastigiata* Waldron: var. *fastigiata*, var. *vulgaris* C. Harz, var. *peruviana* Krapov. & W.C. Gregory and var. *aequatoriana* Krapov. & W. C. Gregory (KRAPOVICKAS and GREGORY 1994).

Cultivated species of several genera have been characterized by cytogenetic techniques in order to detect structural and numerical chromosomic alterations, reconstruct the evolutionary history of the group or assist the construction of genetic maps. Cytogenetic differences between species could be found even in genera with small and similar chromosomes, such as *Citrus* (GUERRA 1993), *Phaseolus* (MOSCONE *et al.* 1999) and *Manihot* (DE CARVALHO and GUERRA 2002).

Arachis chromosomes are small and mostly metacentric making it difficult to find differences between them. HUSTED (1933) distinguished in *A. hypogaea* two pairs of chromosomes different from the others, one of them was smaller and the other had a secondary constriction. HUSTED (1936) also established that *A. hypogaea* is an allopolyploid species. After that, many authors carried out investigations to chromosomically differentiate peanut varieties (D'CRUZ and TANKASALE 1961, SINGH and MOSS 1982, STALKER and DALMACIO 1986, FERNÁNDEZ and KRAPOVICKAS 1994). Up to now, peanut chromosomes have been studied in only four varieties, namely vars. *hypogaea*, *hirsuta*, *fastigiata* and *vulgaris*.

This work reports the chromosomal studies performed in six varieties of *A. hypogaea* to improve the karyological characterization of the varieties, and is aimed at answering the following questions: 1) Is it possible to differentiate subspecies or varieties by means of chromosomal morphology?, 2) Does a relation exist between morphological and chromosomal variation?, 3) Do the chromosomal characteristics contribute to establish the origin of cultivated varieties?

MATERIALS AND METHODS

The nine accessions of *A. hypogaea* and the respective provenance are listed in Table 1. Four accessions belong to subsp. *hypogaea* and five to subsp.

* Corresponding author: Instituto de Botánica del Nordeste, C.C. 209, (3400) Corrientes, Argentina; phone: ++54 3783 422006; fax: ++54 3783 427131; e-mail: lavia@agr.unne.edu.ar.

Table 1 — Karyotypical data. Karyotype formulae, total chromosome lenght (**TCL**), mean length by chromosome (**ML**), mean centromeric index (**CI**), asymmetry intrachromosomic index ($\mathbf{A_1}$), asymmetry interchromosomic index ($\mathbf{A_2}$) and arm ratio (**AR**), SAT chromosomes (type and pair). PC: Primer Catálogo (INTA Manfredi), RCM: Registro Catálogo Manfredi (INTA Manfredi). Collectors: An: V. Anzules, B: D.J. Banks, Ha: L.E. Haro, P: J. Pietrarelli, Vl: F. Valenzuela, Z: H. Zurita.

Taxa	Accessions	Karyotype formulae	TCL	ML	CI	A_1	A_2	AR	SAT chromosomes	
									Type	Pair
subsp. *hypogaea* var. *hypogaea*	PC 558, Guaycurú, Argentina.	36m + 4sm	88.50	2.21 [a]	44,86	0,16	0,60	0,84	5	20
	BP 687, Colorado Rastrero, Ecuador.	36m + 4sm	77.56	1.94 [b]	45,28	0,20	0,55	0,84	5	20
	BPZ 86, Chaucha Morado, Bolivia.	38m + 2sm	76.80	1.92 [b]	46,58	0,11	0,58	0,89	6	20
	RCM 1457, Sopachuy, Bolivia.	38m + 2sm	72,73	1,82 [c]	45,81	0,15	0,61	0,85	5	20
var. *hirsuta*	BPVl 732, Rastrero Morado, Ecuador.	38m + 2sm/st	67.65	1.69 [d]	43,08	0,16	0,56	0,84	6	20
subsp. *fastigiata* var. *fastigiata*	BPZ 684, Rosita, Ecuador.	36m + 4sm	79.04	1.97 [b]	45,18	0,16	0,57	0,83	3	20
var. *æquatoriana*	BPZHaAn 714, Zaruma, Ecuador.	38m + 2sm	72.35	1.81 [c]	45,76	0,15	0,55	0,85	5	20
var. *peruviana*	BPZHaAn 715, Catalán, Perú.	38m + 2sm	72.48	1.81 [c]	45,64	0,15	0,59	0,84	5	20
var. *vulgaris*	cv. Blanco Manfredi 68, Argentina.	38m + 2sm	72,49	1.81 [c]	45	0,18	0,46	0,82	3	20
F(ANOVA) (a=0.001)				21.20**						
P				<0.00						

Values in the column **ML** followed by the same letter are not significantly different.

fastigiata. These accessions are conserved *ex situ* in INTA-EEA-Manfredi (Córdoba-Argentina), and voucher specimens of the analyzed material are deposited at the IBONE herbarium (CTES).

Mitotic preparations were obtained from root-tips of germinating seeds. After a pretreatment for 3 h in a 0,002 M 8-hydroxyquinoline solution at room temperature, the material was fixed in lactic acid-ethanol (1:5) (FERNÁNDEZ 1973) and stained following Feulgen's technique, and then squashed in a drop of 2% acetic orcein.

The following parameters were calculated for the numerical characterization of the karyotypes: total chromosome length (TCL), mean length of the chromosomes (ML), mean centromeric index (CI), arm ratio (AR=(b/B)/n), asymmetry intrachromosomal index (A_1) and asymmetry interchromosomal index (A_2) (ROMERO ZARCO 1986). SAT chromosomes were classified according to FERNÁNDEZ and KRAPOVICKAS (1994).

The karyotypes were described following LEVAN *et al.* (1964) nomenclature. Chromosome morphology was determined by the centromeric index (short arm x 100/total length), and chromosomes were classified as metacentric (m) = 50-37.5, submetacentric (sm) = 37.5-25 and subtelocentric (st) = 25-12.5. Idiograms were constructed based on the mean chromosome length for ten metaphases per sample and arranged by size in decreasing order for each taxon.

Parameter means were compared by one-way ANOVA after Bartlett's test of homogeneity. Turkey's test was carried out to examine karyotype similarity among the species. A data matrix of 9 OTUs (operational taxonomic units) x 8 variables was constructed. The NTSYS-PC program (ROLHF 1994) was used to standardize the data matrix, calculate the average taxonomic distance, and to generate a phenogram. Clustering was performed using the unweighted pair-group method (UPGMA).

RESULTS AND DISCUSSION

The karyotype formulae obtained and the analyzed parameters are summarized in Table 1. Mitotic chromosomes and the respective idiograms are shown in figures 1 and 2, respectively.

All studied *A. hypogaea* accessions were tetraploid (2n=4x=40), and the basic chromosome number was x=10, in agreement with previous reports for the genus (HUSTED 1936, D'CRUZ and TANKASALE 1961, SINGH and MOSS 1982, STALKER and DALMACIO 1986, CAI *et al.* 1987, FERNÁNDEZ and KRAPOVICKAS 1994). Varieties *peruviana* and *ae-*

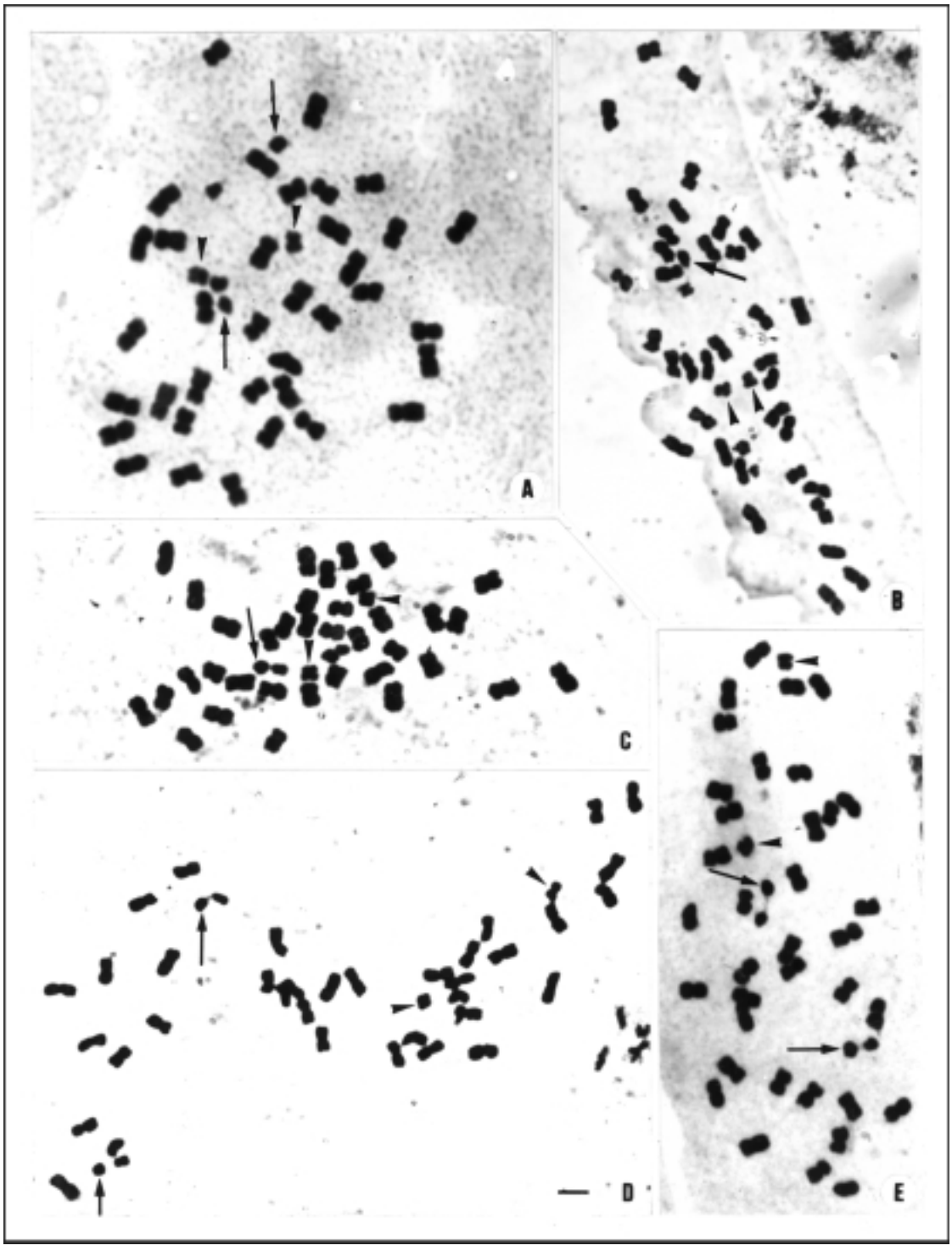

Fig. 1 — Mitotic chromosomes of *A. hypogaea*. A, var. *hypogaea* "Guaycurú"; B, var. *hirsuta* "Rastrero Morado"; C, var. *peruviana* "Catalán"; D, var. *aequatoriana* "Zaruma"; E, var. *vulgaris* cv. Blanco Manfredi. Arrows show satellites and arrowheads "A" chromosomes. Bar=10 µm.

quatoriana were analyzed for the first time in this work.

In general, the average chromosome size found was 1.88 µm ranging from 0.92 to 2.80 µm; similar values were found sixty years ago by HUSTED (1936), varying between 0.80 and 2.70 µm; however, STALKER and DALMACIO (1986) and CAI et al. (1987) found different values, between 1.49 and 4.16 µm.

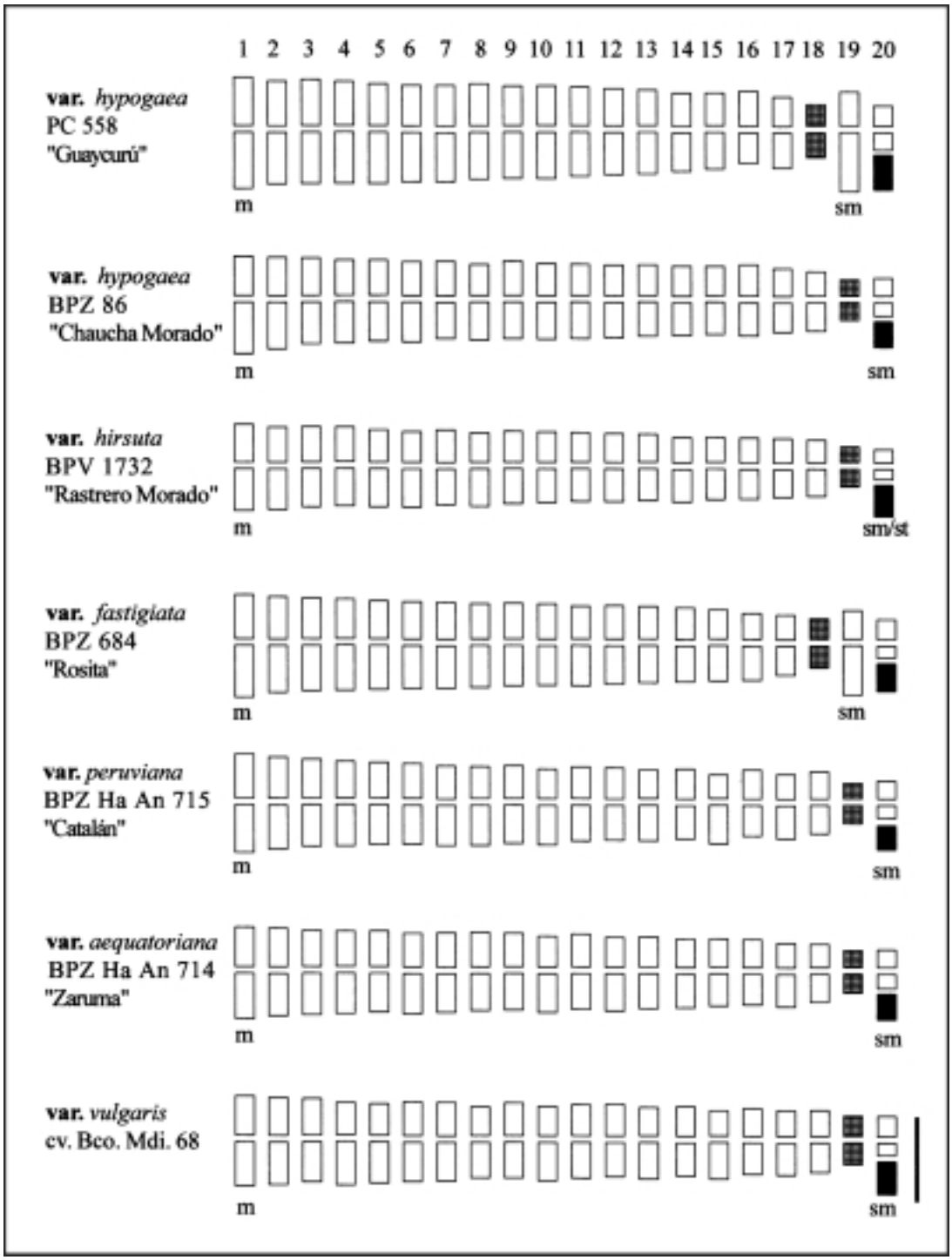

Fig. 2 — Idiograms of *A. hypogaea*. ■ Satellite. ▦ "A" chromosomes. Bar= 2 μm.

According to the figures they presented, the analyzed cells were in prometaphase, whilst we studied cells in metaphase.

The only parameter that showed significant differences was the mean chromosome length, for which four groups were formed (Table 1). No variation was found between vars. *aequatoriana*, *peruviana* and *vulgaris*, since they showed the same mean length.

Regarding the subspecies, the highest variation was observed within subsp. *hypogaea*, ranging between 1.69 μm in "Colorado Rastrero" and 2.21 μm

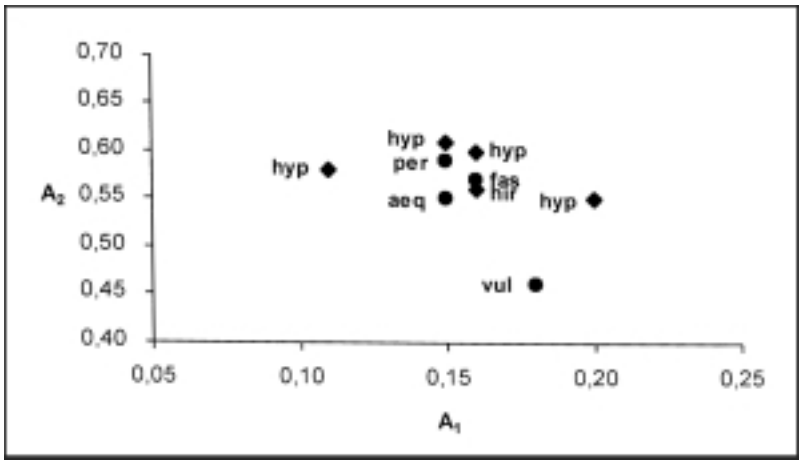

Fig. 3 — Scatter diagram showing the relation between intrachromosomal (A_1 and interchromosomal (A_2) asymmetry indices. Values of A_1 and A_2 are summarized in Table 1. ◆ Subsp. *hypogaea*, ● Subsp. *fastigiata*. Abbreviations: hyp: var. *hypogaea*, hir: var. *hirsuta*, fas: var. *fastigiata*, aeq: var. *aequatoriana*, per: var. *peruviana*, vul: var. *vulgaris.*

in "Guaycurú" (subspecies mean length: 1.91 μm). For subsp. *fastigiata*, the mean chromosome length ranged from 1.81 μm in vars. *aequatoriana*, *peruviana* and *vulgaris* to 1.97 μm in var. *fastigiata* (subspecies mean length:1.85 μm). There were no significant differences between the subspecies mean length. However, differences between varieties were observed, showing that var. *hirsuta* was distinctive for having the smallest chromosome length (1.69 μm).

Significant intravarietal differences were found within var. *hypogaea*. Four accessions of subsp. *hypogaea* var. *hypogaea* from different countries were studied. This varieties presented the greatest chromosome length variation and three groups were defined: 1) "Guaycurú" (2.21 μm), 2) "Colorado Rastrero" and "Chaucha morada" (1.93 μm), 3) "Sopachuy" (1.82 μm) (Table 1).

In *Arachis* genus, sections have been characterized by the SAT chromosome type (LAVIA *et al.* 2001). In this work, all the accessions in mitotic metaphases exhibited a pair of satellites. FERNÁNDEZ and KRAPOVICKAS (1994) reported SAT type 5 in var. *hirsuta* and SAT type 3 for var. *fastigiata*. In the present work, we analysed more accessions and observed SAT type 6 for var. *hirsuta* and type 3 for var. *fastigiata* (Fig. 2). Moreover, we detected SAT types 5 and 6 in var. *hypogaea*, SAT type 3 in var. *vulgaris* and SAT type 5 in var. *aequatoriana* and *peruviana*. In conclusion, SAT chromosome type 3 was only observed in subsp. *fastigiata* while the SAT chromosome type 6 was exclusive for subsp. *hypogaea*.

The studied accessions had similar karyotype formulae (Table 1). Most of the chromosomes were m and one or two pairs sm (Fig. 1, 2), except in subsp. *hypogaea* var. *hirsuta*, which presented one pair sm/st that correspond to the SAT chromosomes. For both subspecies, HUSTED (1933) and FERNÁNDEZ and KRAPOVICKAS (1994) noticed karyotype formulae 38m+2sm. In this work, the accessions of subsp. *hypogaea* presented the karyotype formulae: 36m+4sm, 38m+2sm and 38m+2sm/st, whilst the subsp. *fastigiata* showed two formulae: 38m+2sm in three varieties and 36m+4sm in one variety.

According to the mean centromeric index, the studied accessions fall into the metacentric category. The A_1 and A_2 indices are plotted in a scatter diagram (Fig. 3). The diagram shows that the karyotypes of *hypogaea* varieties present the greatest variation in A_1 and A_2 values. On the other hand, *fastigiata* varieties are more homogeneous, except for var. *vulgaris* that has the lower A_2 (0.46). Statistical significant differences were not found for A_1 and A_2 indices, confirming that the karyotypes are highly symmetric.

Cluster analysis of karyological data is presented in Figure 4. Four clusters could be recognized among the accessions studied. Variety *hirsuta* ("Rastrero Morado"), which conformed the first cluster, possesses a distinctive karyotype formulae (38m+2sm/st) and the shortest mean chromosome length. The chromosome SAT type separated the other three groups. One of them was made up by var. *fastigiata* ("Rosita") and var. *vulgaris* ("cv. Blanco Manfredi"), which have SAT chromosome type 3, the other by var. *hypogaea* ("Chaucha Morado") with SAT chromosome type 6. The last cluster, with SAT chromosome type 5, comprises two sub-clusters: one with var. *hypogaea* ("Guaycurú" and "Colorado rastrero") which present the same karyotype formulae 36m+4sm, and other with var. *hypogaea* ("Sopachuy"), var. *aequatoriana* ("Zaruma") and var. *peru-*

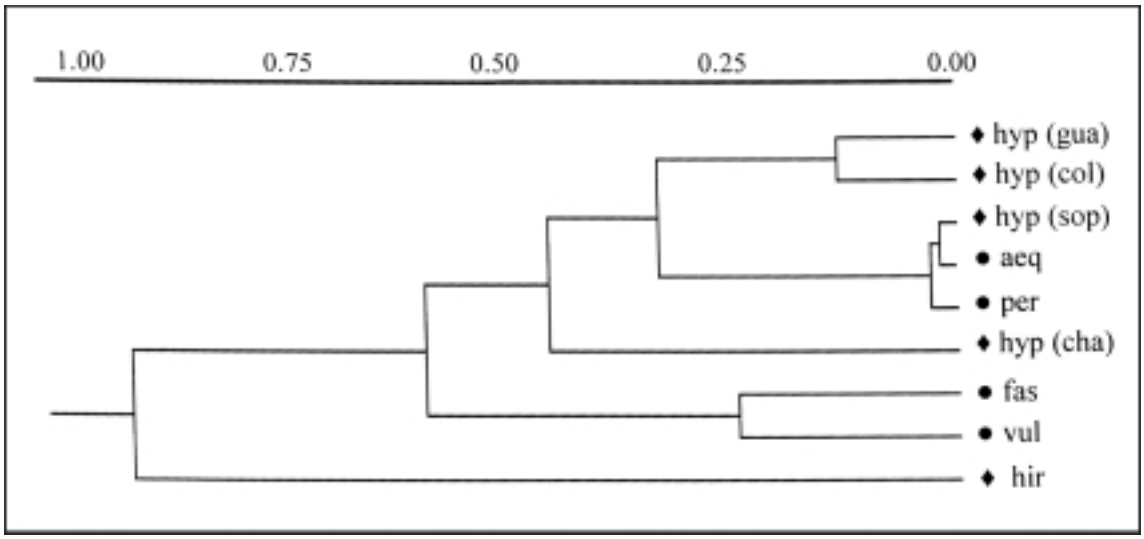

Fig. 4 — Phenogram of unweighted-pair groups method (UPGMA) derived from average taxonomic distance. ♦ Subsp. *hypogaea*, ● subsp. *fastigiata*. Abbreviations: hyp: var. *hypogaea*, hir: var. *hirsuta*, fas: var. *fastigiata*, aeq: var. *aequatoriana*, per: var. *peruviana*, vul: var. *vulgaris*, gua: "Guaycurú", col: "Colorado Rastrero", sop: "Sopachuy", cha: "Chaucha Morado".

viana ("Catalán"), which possess 38m+2sm and nearly equal chromosome length.

It is important to point out that *peruviana* and *æquatoriana* varieties were very close in the phenogram since all the analyzed characteristics in these varieties were very similar.

The phenogram and scatter diagram analysis showed that the accessions are not grouped by subspecies. This indicates that chromosomal features did not allowed to separate the *hypogaea* subspecies. Our results, in spite of morphological variation described between these varieties (KRAPOVICKAS and GREGORY 1994), showed that similar karyotypes are present within the *A. hypogaea*. STEBBINS (1971) suggested that similar karyotypes could exist in a species highly diverse while a genus morphologically and ecologically homogeneous could have a great variety of karyotypes.

The analysis of chromosome morphology by classical techniques (present work), physical mapping of 5S and 18S-25S rRNA genes by FISH (SEIJO *et al.* 2004), and genetic variability by RFLP and RAPD markers (HALWARD *et al.* 1991, KOCHERT *et al.* 1991) showed that the *A. hypogaea* varieties are chromosomally and genetically very similar. Regarding the origin of *A. hypogaea*, our results supports the hypothesis that suggest a monophyletic origin.

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